



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 39/395, 48/00, C07K 16/30, 16/18	A1	(11) International Publication Number: WO 98/56416 (43) International Publication Date: 17 December 1998 (17.12.98)
(21) International Application Number: PCT/IL98/00266 (22) International Filing Date: 9 June 1998 (09.06.98) (30) Priority Data: 121041 9 June 1997 (09.06.97) IL (71) Applicant (for all designated States except US): YEDA RESEARCH AND DEVELOPMENT CO. LTD. [IL/IL]; The Weizmann Institute of Science, P.O. Box 95, 76100 Rehovot (IL). (72) Inventors; and (75) Inventors/Applicants (for US only): COHEN, Irun, R. [IL/IL]; Hankin Street 11, 76354 Rehovot (IL). ROTTER, Varda [IL/IL]; Mivtsa Kadesh 2, 75221 Rishon LeZion (IL). WOLKOWICZ, Roland [IL/US]; Apartment 9, 130 Arch Street, Redwood City, CA 94062 (US). RUIZ, Pedro, J. [CO/US]; Beckman Center B002, Stanford, CA 94305-5429 (US). EREZ-ALON, Neta [IL/IL]; Lea Street 18, 69412 Tel Aviv (IL). HERKEL, Johannes [DE/DE]; Griesaeckerstrasse 3, D-97078 Wuerzburg (DE). (74) Agent: BEN-AMI, Paulina; Yeda Research and Development Co. Ltd., The Weizmann Institute of Science, P.O. Box 95, 76100 Rehovot (IL).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: IMMUNOGENIC COMPOSITIONS FOR INDUCTION OF ANTI-TUMOR IMMUNITY		
(57) Abstract <p>The invention relates to the use of an immunogen selected from the group consisting of: (i) a monoclonal antibody (mAb) to a tumor-associated antigen (TAA); (ii) a fragment of a mAb of (i); (iii) a peptide based on a complementarity-determining region (CDR) of the heavy or light chain of said mAb of (i), which peptide is capable of eliciting antibodies to said TAA; and (iv) a DNA molecule coding for the variable (V) region of said mAb of (i) in a suitable gene delivery vehicle, for the preparation of a pharmaceutical composition useful for induction of anti-tumor immunity in mammals, for activating an enhanced immune response to a TAA molecule in mammals, and/or for induction of immune responses to mutated and wild-type forms of a TAA in mammals. The use of anti-p53 mAbs and novel peptides based on the CDR2 and CDR3 of the heavy chains and CDR3 of the light chains of different anti-p53 mAbs are disclosed.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

IMMUNOGENIC COMPOSITIONS FOR INDUCTION OF ANTI-TUMOR IMMUNITY

FIELD OF THE INVENTION

The present invention relates to the use of an immunogenic composition for the induction of anti-tumor immunity in mammals, said composition comprising a pharmaceutically acceptable carrier and an immunogen selected from the group consisting of : (i) a monoclonal antibody (mAb) to a tumor-associated antigen (TAA), (ii) a fragment of said mAb, (iii) a peptide based on a complementarity-determining region (CDR) of said mAb, and (iv) a nucleic acid sequence coding for the variable region of said mAb, and to a method for induction of anti-tumor immunity in a subject comprising administration of said immunogen. In a particular example, the TAA is p53 and the immunogen is an anti-p53 mAb, a fragment thereof, peptides based on a CDR of said anti-p53 mAb, or a nucleic acid sequence coding for the variable region of said anti-p53 mAb.

ABBREVIATIONS

The following abbreviations will be used herein in the specification and claims:

anti-id - anti-idiotypic; **mAb** - monoclonal antibody; **TAA** - tumor-associated antigen; **CDR** - complementarity-determining region of the heavy or light chain of a mAb.

BACKGROUND OF THE INVENTION

The rejection of transplanted cells and tissues of allogeneic origin proves that the immune system is capable of destroying its targets. It has long been a goal of immunologists to direct the destructive capability of the immune system against a person's tumor cells and thereby effect the rejection of the tumor and the cure of the patient. Immunotherapy would be especially useful to rid a person of residual tumor cells that have spread beyond the site of the primary tumor mass. The primary tumor can usually be resected surgically or irradiated by local radiotherapy. The patient too often succumbs

later, however, to metastatic tumor cells that have spread to other parts of the body. Immunotherapy would be an ideal way to destroy nests of metastatic tumor cells before they grow into large life-threatening tumors. Lymphocytes patrol the tissues and lymphocytes sensitized to the tumor cells could kill the metastatic tumor cells remaining
5 after resection of the primary tumor mass.

The problem for immunotherapy, however, is to activate the immune system against antigens that can mark the tumor cells for destruction. It had been hoped that tumors might bear "foreign transplantation" antigens produced by viruses or created by immunologically significant mutations of genes in the tumor cell. It now turns out that such tumor specific
10 transplantation antigens (TSTA) are rare.

Tumor cells are characterized immunologically for the most part by TAA, which are molecules that are expressed in normal cells too. These TAA may appear normally during the early development of healthy cells, or they may be expressed normally at lower concentrations than in tumor cells. TAA may also feature minor mutations that do not
15 appear "foreign" to the immune system and thus do not stimulate strong immune responses. TAA, for the most part, are self-antigens and, as such, they are not very immunogenic. The immune system is normally tolerant to the body's own antigens. Therefore, the induction of effective immunity against TAA is tantamount to inducing an autoimmune reaction. Immunotherapy of tumors requires the activation of the equivalent
20 of an autoimmune reaction against the tumor cells. Moreover, it is most desirable to have the autoimmune reaction limited to the tumor itself, so the autoimmune reaction terminates once the tumor cells are destroyed. Nevertheless, autoimmunity can be compatible with life, while metastatic cancer can kill. Therefore, residued autoimmunity is a tolerable price to pay for successful tumor immunotherapy.

25 Examples of known TAA include p53 protein, neu differentiation factor (NDF), epidermal growth factor (EGF), carcinoembryonic antigen (CEA), and tyrosinase enzyme.

The p53 protein is the product of a tumor suppressor gene that functions to arrest the growth of mutated or aberrant cells. The p53 protein is a transcription factor that binds specifically to a consensus site present in the regulatory sequences of p53-dependent genes
30 (el-Deiry et al., 1992; Zambetti and Levine, 1993). Mutation of the p53 gene in the domain encoding binding to the specific DNA regulatory site causes a loss of tumor suppression (Zambetti and Levine, 1993). Therefore it is not surprising that a significant

proportion of natural human tumors bear mutated p53 (Hollstein et al., 1991). For reasons that are not entirely clear, tumor cells also appear to accumulate wild-type p53 and not only mutated p53 in their cytoplasm (Moll et al., 1995). Thus the wild-type p53 molecule, and not only the mutated p53 molecule, can serve as a target for a potentially therapeutic
5 anti-tumor immune response.

Inactivation of the p53 tumor suppressor protein by mutation of the gene or by viral insertion, gene rearrangement, or other causes is a common event in human cancers. Point mutation or deletion of the p53 gene is the most common genetic aberration in human neoplasms. Approximately 70% of colon cancers, 30 to 50% of breast cancers, 50% of
10 lung cancers, and almost 100% of small-cell carcinomas of the lung harbor p53 mutations (Hollstein et al., 1991). The development of a tumor is often associated with accumulation in the cancer cells of the p53 protein, wild-type or mutant. Furthermore, mutated p53 proteins are tumor-specific antigens that can be recognized as targets by the immune system (Melief and Kast, 1991; Yanuck et al., 1993). Cancer patients can manifest
15 immune responses directed to wild-type and mutant p53 proteins. The p53 protein, mutant and wild-type, can accumulate in the cytoplasm of cancer cells, and cancer patients have indeed been found to produce antibody (Lubin et al., 1993; Schlichtholz et al., 1992) and T cell responses to p53 (Houbiers et al., 1993; Tilkin et al., 1995). Normal cells express p53 to a much lower degree and, unlike tumor cells, normal cells show no accumulation of p53
20 in the cytoplasm. Thus, tumor cells and normal cells differ in both the amount and compartment of p53 expression. For these reasons, the wild-type p53 molecule, and not only the mutated p53 molecule, can serve as a target for a potentially therapeutic anti-tumor immune response.

To identify T-cell epitopes in p53, Houbiers et al., 1993, synthesized peptides of
25 wild-type p53 and peptides with the point mutations of p53 detected in colorectal and ovarian cancers. Some of the p53 peptides were shown to bind in vitro to HLA-A2.1 molecules and to induce specifically cytotoxic T lymphocytes (CTL) clones. Characterization of anti-p53 immunity and its implications for tumor therapy have been studied using peptides derived from wild-type or mutated p53 sequences to elicit CTL
30 responses in experimental animals (Noguchi et al., 1994; Noguchi et al., 1995; Yanuck et al., 1993). Mouse fibroblasts transfected with a mutated human p53 gene were specifically killed by CD8⁺ CTL from the spleens of mice that had been pulsed with a 21-amino acid

peptide encompassing a p53 point mutation from a human lung carcinoma (Yanuck et al., 1993). A nonapeptide containing a codon 234 mutation (234CM) induced CD8⁺ CTL that lysed a 234CM-pulsed P1HTR mastocytoma cell line (Noguchi et al., 1994). Mice immunized with peptide 234CM were resistant to challenge with Meth A sarcoma cells
5 (Noguchi et al., 1994), and vaccines containing peptide 234CM in the QS-21 adjuvant caused regression of established Meth A tumors in mice treated with IL-12 (Noguchi et al., 1995).

Thus, both mutated p53 and wild-type p53 are tumor-associated antigens and attempts have been made to use these molecules as immunogens for tumor immunotherapy
10 (Houbiers et al., 1993; Noguchi et al., 1994; Noguchi et al., 1995; Yanuck et al., 1993; published PCT Application WO 94/02167). However, p53 is not very immunogenic, probably because it is a self-protein and therefore immunologically tolerated.

An antibody binds to an antigen at its variable region (antigen-binding site). Therefore, the variable regions of antibodies have three-dimensional structures that are
15 complementary to the structures of the antigenic determinants the antibodies recognize.

The binding site of the antibody complementary to the structure of the antigen is created by hypervariable regions of the light and heavy chains of the Fab portion of the antibody. These binding site structures are formed by the collective aggregate of the CDR of the light and heavy chains of the immunoglobulin molecule (Alzari et al., 1988).
20 However, an antibody itself, when recognized by another antibody, can be considered to be an antigen. In the case where structures of the variable regions of the antibody are recognized, these structures are called idiotypes (Id), and the antibodies that recognize the idiotypes of the antibody are called anti-idiotypic (anti-id) antibodies. The structure corresponding to the antigenic determinant of the antibody is called an idiotope (Jerne,
25 1974).

It has been reported that immunization with mAbs can induce immune responses that extend beyond the specificity of the antibody (Takemori et al., 1982), probably by anti-idiotypic connectivity (Jerne, 1974; Cohen, 1989, 1992) based on idiotypic determinants in the variable (V) region of the immunizing mAb (Bruggemann et al., 1989).
30 According to idiotypic antibody network terminology, Ab1 is the first antibody, the antibody binding to the antigen, and Ab2 is the anti-idiotypic antibody to Ab1. The variable region of Ab2 may mimic the conformation of the antigen because both the

antigen and Ab2 can be bound by Ab1. Ab3 is the anti-idiotypic antibody to Ab2. Because of the chain of structural complementarity, Ab1 and Ab3 can have similar specificity for the original antigen.

Antibodies have been used in the past in tumor immunotherapy in two ways: Ab1
5 antibodies as tumor-specific antigens on B lymphoma cells, and Ab2 antibodies as anti-idiotypic mimics of tumor antigens. Ab1 idiotypic determinants expressed by immunoglobulins on the surface of neoplastic B cells have been used in experimental models as tumor-associated targets to induce protective immunity (Ab2) against B cell lymphomas which, unlike solid tumors, are particularly sensitive to antibodies (reviewed
10 by Yefenof et al., 1993). However, Ab1 idiotypic determinants are unique to each B-cell tumor, and the practical requirements of preparing an individual protein vaccine for each patient has made the application to the clinic difficult and expensive (Stevenson et al., 1995).

Ab2 antibodies mimicking TAAs of various kinds have been used to induce
15 antibodies (Ab3) to tumor antigens (reviewed by Wettendorf et al., 1990). However, Ab2 immunization has been usually less successful than has immunization with the TAAs themselves (Wettendorf et al., 1990).

With regard to the possible anti-cancer effects of anti-p53 antibodies, cancer patients have been found to produce antibodies to the amino terminus of the p53 molecule, but
20 these antibodies appear to mark the development of cancer rather than to protect against the disease (Soussi, 1996). Investigation of the effects of immunity to the central and carboxy domains of p53 might therefore be of some importance

Published International PCT Application No. WO 94/12202 describes the activation of a mutant p53 that occurs at elevated levels in tumors and does not substantially suppress
25 tumor growth, for specific DNA binding, wherein the mutant p53 is activated with a ligand capable of binding to, and activating the mutant p53, wherein the ligand may be the anti-p53 mAb 421 which binds to the carboxy terminal region of p53, or the bacterial heat shock protein DnaK, or a ligand which binds effectively to the same site on the mutant p53.

30

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a novel way of inducing effective anti-tumor immunity without apparent harm to the patient.

It has now been found in accordance with the present invention that immunization of BALB/c or C57BL/6 mice with anti-p53 mAbs (Ab1) directed to different domains of the p53 molecule induces spontaneously antibodies to p53 (Ab3), and that anti-DNA antibodies (Ab2) are generated if Ab1 is directed to a DNA-binding domain of p53.

The present invention thus relates to the use of an immunogen selected from the group consisting of:

- (i) a mAb to a TAA;
- (ii) a fragment thereof;
- (iii) a peptide based on a CDR of the heavy or light chain of said mAb of (i), which peptide is capable of eliciting antibodies to said TAA; and
- (iv) a DNA molecule coding for the variable (V) region of the mAb of (i) in a suitable gene delivery vehicle,

for the preparation of a pharmaceutical composition useful for induction of anti-tumor immunity in mammals, for activating an enhanced immune response to a TAA molecule in mammals, and/or for induction of immune responses to mutated and wild-type forms of a TAA in mammals.

In another aspect, the present invention relates to methods for induction of anti-tumor immunity in a mammal, for activating an enhanced immune response to a TAA in a mammal, and/or for induction of immune responses to mutated and wild-type forms of a TAA in a mammal, which comprises administering to said mammal an effective amount of an immunogen selected from the group consisting of:

- (i) a mAb to a TAA;
- (ii) a fragment thereof;
- (iii) a peptide based on a CDR of the heavy or light chain of said mAb of (i), which peptide is capable of eliciting antibodies to said TAA; and
- (iv) a DNA molecule coding for the variable (V) region of the mAb of (i) in a suitable gene delivery vehicle.

The TAA according to the invention may be any TAA including, but not being limited to, p53 protein, neu differentiation factor (NDF), epidermal growth factor (EGF), carcinoembryonic antigen (CEA) and tyrosinase enzyme.

5 The anti-TAA mAb used according to the invention may be directed against different domains of an anti-wild type TAA or an anti-mutant TAA mAb, and may be a murine, human or humanized anti-wild type or anti-mutant TAA mAb.

The anti-TAA mAb fragments that can be used according to the invention include antigen-binding fragments (Fab), F(ab')₂ or any other type of antibody molecule, including single chain Fv fragments of antibodies, as long as such antibody fragments are able to
10 bind the TAA, as well as peptides based on a CDR of the heavy or light chain of an anti-TAA mAb, which peptides are capable of eliciting antibodies to the TAA without necessarily binding said TAA.

In one embodiment of the present invention, the TAA is the p53 molecule, and thus the invention relates to the use of an immunogen selected from the group consisting of:

- 15 (i) an anti-p53 mAb;
(ii) a fragment of an anti-p53 mAb;
(iii) a peptide based on a CDR of the heavy or light chain of an anti-p53 mAb, which peptide is capable of eliciting antibodies to p53; and
(iv) a DNA molecule coding for the variable (V) region of an anti-p53 mAb in a
20 suitable gene delivery vehicle,
for the preparation of pharmaceutical compositions useful for induction of anti-tumor immunity in mammals, for activating an enhanced immune response to the p53 molecule in mammals, and/or for induction of immune responses to mutated and wild-type forms of p53 in mammals.

25 In another embodiment, the present invention relates to a method for induction of anti-tumor immunity in a mammal, or for activating an enhanced immune response to the p53 in a mammal, or for induction of immune responses to mutated and/or wild-type forms of p53 in a mammal, said method comprising immunizing said mammal with an effective amount of an immunogen selected from the group consisting of:

- 30 (i) an anti-p53 mAb;
(ii) a fragment thereof;

- (iii) a peptide based on a CDR of the heavy or light chain of an anti-p53 mAb, which peptide is capable of eliciting antibodies to p53; and
- (iv) a DNA molecule coding for the V region of an anti-p53 mAb in a suitable gene delivery vehicle.

5 In still a further aspect, the present invention relates to a method for the generation of sequence-specific anti-DNA antibodies which comprises immunizing a mammal with a mAb directed to a domain containing a DNA-binding site of a DNA-binding protein, e.g. p53, and recovering the thus elicited sequence-specific anti-DNA antibodies.

10 **BRIEF DESCRIPTION OF THE DRAWINGS**

Fig. 1 shows the retardation in gel of the p53-specific oligonucleotide sequence by anti-DNA antibodies developed in mice immunized with anti-p53 mAb 246, but not with mAb 240, mAb 421, mAb 9E10 and mAb R73.

15 Figs. 2A-2B show immunoprecipitation of labeled p53 produced by reaction of an extract of Meth A tumor cells, metabolically labeled with ³⁵S-methionine, with anti-p53 mAbs 421, 246 and 240 (Fig. 2A) or with antisera induced by immunization with mAbs 421, 240 (Fig. 2A), or mAb 246 and control 9E10 (Fig. 2B).

Fig. 3 shows the sequences of the heavy (VH) and light (VL) chains of the anti-p53 mAbs 240, 246, 248 and 421, in which the CDR sequences are underlined.

20 Fig. 4 shows the protective effect of immunizing mice with the anti-p53 mAbs 240, 246 and 421 against the development of lung carcinoma metastasis in mice. mAb R73 was used as a control immunogen. Dots indicate the weight of individual lungs, bars the median of each group. Normal lung weight is around 200 mg.

25 **DETAILED DESCRIPTION OF THE INVENTION**

The present invention relates, in one aspect, to the use of an Ab1 anti-TAA mAb, e.g. anti-p53 mAb, to generate an Ab3 anti-TAA, e.g. anti-p53, response. Presumably, the chain reaction from Ab1 to Ab3 implies the generation of Ab2 antibodies as intermediates.

30 To activate an enhanced immune response to p53 according to the invention, an anti-idiotypic network (reviewed by Nisonoff, 1991) inducible by immunization with antibodies as immunogens rather than with antigens, was exploited.

The p53 molecule has two attributes of immunological interest: (a) because p53 binds DNA, immunity to p53 may lead to anti-DNA antibodies by an anti-id network: antibodies to a DNA-binding site of p53 can mimic DNA and, therefore, such anti-p53 antibodies might induce anti-DNA antibodies as anti-ids; and (b) because p53 accumulates
5 in transformed cells, immunity to p53 may have an anti-tumor effect.

The generation of antibodies to DNA has been difficult because the DNA molecule is poorly immunogenic. In particular, it would be desirable to obtain antibodies to specific DNA sequences as such antibodies can be used to detect the presence of such sequences for purposes of diagnosing whether an individual has a specific gene or promoter
10 sequence. A specific antibody would have an advantage over currently used PCR (polymerase chain reaction) techniques, because antibody binding, unlike the PCR, is easily quantifiable and needs no primers or enzymatic replication. In particular, sequence-specific anti-DNA antibodies can be used in diagnostics, for example, in detecting critical sequences in the breeding of animals and plants, in the identification of
15 bacteria and other parasites, in determination of paternity and maternity, in forensic medicine. Specific anti-DNA antibodies also can be useful in the isolation of specific genes for DNA vaccination, gene cloning, and gene sequencing. Antibodies to specific sequences of DNA might also have a use in the activation or inhibition of particular genes for therapeutic purposes in plants, animals or humans. It has been shown that antibodies
20 penetrate into living cells (Alarcon-Segovia et al., 1996; Madaio et al., 1996; Yanase et al., 1994; Ma et al., 1991) and anti-DNA antibodies might be able to exert effects within living cells. But DNA in general, and certainly specific sequences of mammalian DNA, are not immunogenic.

According to the present invention, the p53 molecule was used as model of a
25 DNA-binding protein in order to learn whether sequence-specific anti-DNA antibodies might be generated by an anti-id network centered around immunity to a DNA-binding protein. The p53 molecule might serve as a model of a well-characterized DNA-binding protein that recognizes a highly specific p53 regulatory site (el-Deiry et al., 1992). This domain makes it possible for the p53 molecule to bind to the p53-specific DNA regulatory
30 site, leading to growth arrest of the aberrant cell. Antibodies to the specific DNA-binding site of p53 can be used to determine whether specific anti-DNA antibodies (as Ab2) can be generated, by way of an anti-id network, using antibodies to the specific DNA-binding

domain of p53 (as Ab1). Because of the structural similarity between DNA and RNA, it is obvious that the specific anti-DNA antibodies could also recognize specific RNA.

Induction of an immune response to specific DNA (Ab2) and to mutated and wild-type forms of p53 (Ab3) is provided according to the invention by immunization with mAbs (Ab1) specific for particular domains of wild-type or mutated p53 proteins. The
5 induction of anti-p53 antibodies (Ab3) is hereon shown to be associated with the development of resistance to challenge with a murine fibrosarcoma tumor Meth A and with inhibition of lung metastases produced by the spontaneously metastasizing 3LL tumor. Since over-expression and/or accumulation of p53 antigen is common to many tumors,
10 immunization with antibodies directed to the p53 protein, by eliciting an idiotypic network response, may be useful in the development of a general therapy for different cancers, particularly since effector T cells are activated in such networks.

The induction of specific anti-DNA antibodies by immunization with a mAb to the domain of p53 that binds specific DNA indicates that anti-DNA antibodies can indeed
15 arise by an anti-id network. Thus, the anti-id network appears to preserve structural similarity with particular p53 domains.

Thus, in particular embodiments of the present invention, immunization either with an anti-mutant p53 mAb such as mAb 240 that is specific for a mutated p53 conformation (Gannon et al., 1990), or with an anti-wild type p53 mAb such as mAb 246 that is specific
20 for the native conformation of p53, at the edge of the DNA-binding site of native p53 (Yewdell et al., 1986), or with a mAb against both the wild-type and mutant p53, such as mAb 421 that is specific for the carboxy terminal domain of p53 conserved in both wild-type and mutant p53 (Arai et al., 1986), induced anti-p53 antibody titers both in BALB/c and in C57BL/6mice, apparently by way of an anti-idiotypic network. This
25 anti-p53 immunity was associated with the acquisition of the ability to reject both primary tumors and metastatic tumors.

However, the development of specific anti-DNA antibodies (Ab2) varied with the domain of p53 recognized by the Ab1 used to activate the network. Both the mAb 240 specific for mutated p53, and the mAb 421 specific for the carboxy terminus, did not
30 induce sequence-specific anti-DNA antibodies. However, the mAb 246 induced antibodies to the p53-specific DNA sequence. Thus, sequence-specific anti-DNA antibodies can be produced by immunization with anti-p53 mAbs specific to the central DNA-binding

domain of p53, such as mAb 246; and generation of anti-DNA antibodies can be avoided by the use of an anti-p53 antibody, such as mAb 240, that does not bind a domain of p53 that binds to DNA.

The present invention encompasses the use of any anti-TAA mAb, most preferably any anti-p53 mAb, or a fragment thereof. Since different mutations of p53 may generate a common mutant conformation (Gannon et al. 1990), it may not be necessary to tailor a mAb to each mutation of p53. Thus, according to the present invention, anti-p53 mAbs to either native or mutant p53 can serve as immunotherapeutic agents of wide applicability in the treatment of cancer. The therapeutic anti-p53 mAbs can be of various kinds, including murine, human, or "humanized" mouse antibodies, all of them isolated or prepared in different ways by standard procedures.

Anti-TAA, particularly anti-p53, mAb fragments according to the invention include antigen-binding fragments (Fab), F(ab')₂ or any other type of antibody molecule, including single chain Fv fragments of antibodies, as long as such antibody fragments are able to bind to the TAA, e.g. p53.

The use of genetically engineered humanized mAb or of mAb fragments may be preferred according to the invention because they minimize the amount of foreign protein to be injected and avoid an immune response that may be generated against a murine mAb.

The structural correlates of idiotopes that define the unique binding specificities of antibodies include the CDR hypervariable segments of the immunoglobulin heavy and light chains (Alzari et al., 1989). Thus, according to the present invention, synthetic peptides containing the sequence of a CDR of the heavy or light chain of an anti-TAA, most preferably anti-p53 mAb, and not only the intact mAb molecule, induce anti-p53 immunity by the anti-idiotypic network.

The invention thus further relates to synthetic peptides based on a CDR of the heavy or light chain of an anti-TAA, particularly anti-p53, mAb, and salts and chemical derivatives thereof. Examples of such peptides are peptides containing one or more of the sequences of the CDR2 and CDR3 of the heavy chain and of the CDR3 of the light chain of the mAbs against p53, which sequences are underlined in Fig. 3 as follows:

(i) Peptides, herein designated Ia-Ib, based on the CDR2 and CDR3, respectively, of the heavy chain (240VH), and peptide Ic based on the CDR3 of the light chain (240VL), of the anti-p53 mAb 240, of the sequences:



(Ia) Glu-Ile-Asp-Pro-Ser-Asp-Ser-Tyr-Thr-
Asn-Tyr-Asn-Gln-Asn-Phe-Lys-Asp

(Ib) Leu-Leu-Arg-Tyr-Phe-Ala-Met-Asp-Tyr

(Ic) Gln-His-Ile-Arg-Glu-Leu-Thr-Arg

- 5 (ii) Peptides, herein designated IIa-IIb, based on the CDR2 and CDR3, respectively, of the heavy chain (246VH), and peptide IIc based on the CDR3 of the light chain (246VL), of the anti-p53 mAb 246, of the sequences:

(IIa) Asp-Ile-Asn-Pro-Asn-Asn-Gly-Tyr-Thr-
Ile-Tyr-Asn-Gln-Lys-Val-Lys-Gly

10 (IIb) Gly-Gly-Gly-Leu-Lys-Gly-Tyr-Pro-Phe-Val-Tyr

(IIc) Gln-Gln-Arg-Ser-Ser-Phe-Pro-Phe-Thr

- (iii) Peptides, herein designated IIIa-IIIb, based on the CDR2 and CDR3, respectively, of the heavy chain (248VH), and peptide IIIc based on the CDR3 of the light chain (248VL), of the anti-p53 mAb 248, of the sequences:

15 (IIIa) Asp-Ile-Tyr-Pro-Asn-Asn-Gly-Phe-Thr-
Thr-Tyr-Asn-Gln-Lys-Phe-Lys-Gly

(IIIb) Ser-Gly-Ser-Arg-Phe-Asp-Tyr

(IIIc) Gln-Gln-Ser-Asn-Ser-Trp-Pro-Val-His-Ala

- (iv) Peptides, herein designated IVa-IVb, based on the CDR2 and CDR3, respectively, of the heavy chain (421VH), and peptide IVc based on the CDR3 of the light chain (421VL), of the anti-p53 mAb 421, of the sequences:

(IVa) Trp-Ile-Asp-Pro-Glu-Asn-Gly-Asp-Thr-
Glu-Tyr-Ala-Pro-Lys-Phe-Gln-Gly

(IVb) Tyr-Gly-Asp-Ala-Leu-Asp-Tyr

25 (IVc) Trp-Gln-Gly-Thr-His-Ser-Pro-Leu-Thr

- A "chemical derivative" of a peptide of the present invention, as defined herein, contains additional chemical moieties not normally a part of the peptide. Covalent modifications of the peptides are included within the scope of the invention. Such modifications may be introduced into the molecule by reacting targeted amino acid residues of the peptide with an organic derivatizing agent that is capable of reacting with selected side chains or terminal residues. Such derivatives include, but are not limited to,
- 30

esters, N-acyl derivatives, and the like. Many such chemical derivatives and methods of making them are well known in the art.

Also included in the scope of the invention are salts, both organic and inorganic, of the CDR-based peptides.

5 The peptides according to the invention have preferably 9-30 amino acid residues, examples of which are the 17- to 21-mer peptides V, VI, VII, VIII and IX, which are based on the CDR sequences mentioned above as follows: peptide V includes the sequence Ic of the CDR3 of the light chain of mAb 240, peptide VI includes the sequence IVc of the CDR3 of the light chain of mAb 421, peptide VII includes the sequence IIa of the CDR2 of
10 the heavy chain of mAb 246, peptide VIII includes the sequence IIIb of the CDR3 of the heavy chain of mAb 248, and peptide IX includes the sequence IIIc of the CDR3 of the light chain of mAb 248.

Peptides V to IX have the following sequences:

Peptide V: Tyr-Tyr-Cys-Gln-His-Ile-Arg-Glu-
15 Leu-Thr-Arg-Ser-Glu-Gly-Gly-Pro-Ser
Peptide VI: Gly-Val-Tyr-Tyr-Cys-Trp-Gln-Gly-Thr-His-
Ser-Pro-Leu-Thr-Phe-Gly-Ala-Gly-Thr-Lys
Peptide VII: Gly-Asp-Ile-Asn-Pro-Asn-Asn-Gly-Tyr-Thr-Ile-
Tyr-Asn-Gln-Lys-Val-Lys-Gly-Lys-Ala
20 Peptide VIII: Ala-Val-Tyr-Tyr-Cys-Ala-Arg-Ser-Gly-Ser-
Arg-Phe-Asp-Tyr-Trp-Gly-Glu-Gly-Thr-Thr
Peptide IX: Val-Tyr-Phe-Cys-Gln-Gln-Ser-Asn-Ser-Trp-
Pro-Val-His-Ala-Arg-Gly-Gly-Gly-Thr-Lys

The present invention also provides a DNA sequence coding for the variable region
25 of a mAb to a TAA, e.g. p.53, in a suitable gene delivery vehicle. Any gene delivery vehicle such as viral vectors, e.g. recombinant retroviral vectors and recombinant adenovirus vectors, naked DNA nucleic acid molecules such as plasmids, liposomes, bacterial and eukaryotic cells, as well as other suitable vehicles as described in Vile and Russell, 1994, and in published PCT Application WO 96/21015, both references herein
30 incorporated by reference, can be used according to the invention.

According to the invention, the immunogens can be used for treatment of primary, as well as metastatic, tumors of any kind, including but not being limited to brain, breast,

ovarian, uterus, skin, lung, esophagus, colon, prostate, kidney and bladder cancers, leukemias and lymphomas.

A pharmaceutical composition according to the invention comprising an anti-TAA mAb, a fragment thereof or a peptide based on a CDR region of the heavy or light chain of an anti-TAA mAb, together with a pharmaceutically acceptable carrier, can be administered through various routes known in the art, such as oral, intranasal, intravenous, subcutaneous, intramuscular, intraperitoneal, transdermal or other known routes including the enteral route. In a preferred embodiment, the TAA is p53 and the composition comprising the anti-p53 mAb, fragment or CDR-based peptide, is administered subcutaneously.

When the immunogen is a DNA molecule coding for the variable region of an anti-TAA mAb, it is administered in a suitable gene delivery vehicle as described (Vile and Russel, 1994; WO 96/21015).

The dosage of the immunogen to be administered will depend on the type of compound used - a mAb, a fragment or a peptide, and upon the age, sex, weight and condition of the recipient. The doses should not be so large as to cause adverse side effects such as unwanted cross-reactions, generalized immunosuppression, anaphylactic reactions and the like.

It is also encompassed by the present invention to administer to a patient effective amounts of two or more different mAbs directed to a same TAA or to different TAAs, or fragments thereof. The different mAbs or fragments thereof may be administered concomitantly or sequentially at different intervals.

It is further encompassed by the present invention to administer to a patient effective amounts of two or more different peptides based on the same or different CDR regions of the same mAb or of different mAbs directed to the same TAA or to different TAAs. The different peptides may be administered concomitantly or sequentially at different intervals.

Having now generally described the invention, the same will be more readily understood through reference to the following examples.

Although the examples describe experiments wherein the immunogen is an anti-p53 mAb or peptide based on a CDR of an anti-p53 mAb, it should be understood that these examples are provided by way of illustration only, and are intended to encompass any TAA and not to limit the present invention to p53 only.

EXAMPLES

Materials and Methods

(i) Mice. (a) Female mice of the BALB/c strain were obtained from Olac, Oxon, UK, and used at the age of 8-10 weeks. (b) Female mice of the inbred strain C57BL/6 were used at the age of 6-10 weeks. The mice were bred and kept at the animal breeding facilities of the Weizmann Institute of Science, Rehovot.

(ii) Monoclonal antibodies (mAb). The following mouse monoclonal antibodies (all IgG1/ κ) were used: mAb 240, specific for a mutated non-DNA binding conformation of the p53 molecule (Gannon et al., 1990); mAb 246, specific for the native conformation of p53 at the edge of the p53-specific DNA binding site (Cook and Milner, 1990; Yewdell et al., 1986); mAb 421, specific for the carboxy domain of p53 (Arai et al., 1986) and the anti-myconco-protein antibody mAb 9E10 (Munro and Pelham, 1986), used as a control antibody. R73 is a mouse-anti rat-T cell receptor monoclonal antibody (IgG1)(Hunig et al., 1989) and was used as a control antibody.

The antibodies were obtained by growing the hybridomas as ascites in BALB/c mice, collecting the ascitic fluids and purifying the antibodies by protein A affinity chromatography followed by dialysis in PBS.

20

(iii) Tumor cells.

(a) The Meth A tumor is a transplantable 3-methylcholantrene-induced sarcoma of BALB/c origin (Old et al., 1962) that has three mutations in the p53 coding sequence (Arai et al., 1986). The cells were grown in DMEM containing 10% heat-inactivated fetal calf serum (FCS), and passaged through BALB/c mice to select for tumorigenicity. For challenge, mice were injected intradermally with 10^6 Meth A cells. Tumor growth was observed for eight weeks. Rejection was scored as the disappearance of the tumor mass within 20 days after tumor challenge.

(b) The 3LL-tumor (Eisenbach et al., 1983) is a metastasing lung carcinoma cell line of C57BL/6 origin, that constitutively overexpresses p53, mostly wild-type. The cells were grown in DMEM containing 10% heat-inactivated fetal calf serum (FCS).

(iv) Immunization. Mice were immunized intradermally in the hind footpads with 20 µg of mAb in PBS in a 100 µl emulsion 1:1 in Complete Freund's Adjuvant (CFA; Difco Laboratories, Detroit, MI). An intradermal booster injection with 20 µg of the mAb in PBS alone was given three weeks later. Mice were bled at different times after the boost,
5 and the sera were tested for specific antibodies.

(v) Recombinant p53 proteins. *E. Coli* BL21 (DE3) cells transformed with the T7 expression vector containing regularly spliced mouse p53 cDNA were used to express wild type or mutant p53 as previously described (Shohat-Foord et al., 1991; Wolkowicz et al.,
10 1995). For protein induction, IPTG at a final concentration of 0.4 mM was added when the cells had reached an O.D.₆₀₀ of 0.8-1.0. Three hours after induction, the cells were harvested from a 200 ml culture by centrifugation, and the pellet was resuspended in 5 ml of ice-cold lysis buffer (20mM Tris-HCl, pH: 7.4; 500 mM NaCl; 10% glycerol; 1mM EDTA; 1mM PMSF; 5µg/ml leupeptine; 10 µg/ml aprotinin and 0.1% NP40). The lysate
15 was subjected to sonication for 10 cycles of 15 s of sonication followed by 15 s of gentle swirling. The solicited sample was subjected to two cycles of freeze-thaw and then centrifuged for 10 min at 10.000 x g. The supernatant was diluted with an equal volume of ice-cold water and an equal volume of saturated ammonium sulfate was added drop-wise to obtain a final concentration of 33% saturated ammonium sulfate. After 15 min on ice
20 and centrifugation, the pellet was resuspended in 1 ml of lysis buffer and centrifuged for 5 min at 4° C. The supernatant was diluted with 4 ml of lysis buffer devoid of NaCl. Wild type p53 and mutant p53 were translated *in vitro* using Bluescript plasmids carrying the specific cDNA (Promega, Madison, WI).

25 (vi) Antibody assays. Antibodies to DNA or to p53 were detected using three different assay systems. (i) immunoprecipitation was used to detect antibodies to wild-type and mutant p53, as described (Wolkowicz et al., 1995; (ii) gel-retardation was used to detect antibodies to the synthetic oligonucleotide consensus sequence specific for p53 (Wolkowicz et al., 1995); and (iv) ELISA was done according to Waisman et al., 1993,
30 using anti-mouse IgG and IgG1, IgG2a and IgG2b specific reagents to detect IgG antibody isotypes (Elias et al., 1997).

(vii) Cloning and sequencing of immunoglobulin H and L chains. The polymerase chain reaction (PCR) was used to amplify mAb cDNA as described (Sambrook et al., 1989). Briefly, forward primers located in the constant region or in the J region and backward primers located in the V region, were used for amplification using Taq DNA polymerase (Promega, Madison, WI, USA). The primers used for the PCR were described by Orlandi et al., 1989.

The PCR product was cloned, and ligated DNA was inserted into competent bacterial cells. Positive colonies were picked using specific internal primers that were labeled with γ -ATP. Positive clones were grown and ssDNA was prepared as described (Sambrook et al., 1989). The ssDNA was sequenced using the Sequenase 2.0 kit (USB).

PCR products were separated from the primers used for the reaction using Primer Remover (Advanced Genetic Technologies, MD, USA). Around 1200 ng DNA were used for each sequencing (Applied Biosystems). Sequences were analyzed using Applied Biosystems and GCG package programs.

(viii) Peptide synthesis. Peptides were synthesized as described (Konen-Waisman et al., 1995). Briefly, peptides were prepared with an automated multiple peptide synthesizer (Abimed Model AMS 422; Langenfeld, Germany) using the company's protocols for N- α -fluorenylmethoxycarbonyl (Fmoc) synthesis, or were prepared manually by a standard solid phase method (Merrifield, 1963) employing either N- α -Fmoc or N- α -t-butyloxycarbonyl (t-Boc) strategies. Crude products were purified by reversed phase HPLC on a semi-preparative C8-column (Lichrosorb RP-8, 7 μ m, 250x10 mm, Merck, Darmstadt, Germany). Elution of peptides was achieved by linear gradients established between 0.1% trifluoroacetic acid (TFA) in water and 0.1% TFA in 75% acetonitrile in water (v/v). The purity of the single peptide products was ascertained by analytical reversed-phase HPLC and amino acid analysis.

EXAMPLE 1. Induction of anti-p53 immunity

To exploit the idiotypic network to induce immunity to the p53 protein, BALB/c mice were unimmunized or were immunized with control mAb 9E10, or with anti-mutant p53 mAb 240 or anti-native p53 mAb 246. Ten days after the boost, the mice were bled

and the sera were tested for antibodies to native p53 or mutated p53 by an ELISA assay, or by a precipitation assay. Antibodies were measured after booster immunization.

For the ELISA assay flat bottom maxi-sorb plates (Nunc, Roskilde, Denmark) were coated with 50 μ l per well of mutant or wild type p53 at a concentration of 10 μ g/ml. After incubation with antigen, the plates were washed and blocked over night with 5% fetal calf serum (FCS; BioLab, Jerusalem, Israel) or with 1% bovine serum albumin (BSA; Sigma) in PBS. Test sera diluted serially (1/50 to 1/800) were then added for 90 min, followed by incubation for 75 min with 50 μ l per well of alkaline phosphatase-conjugated goat anti-mouse IgG, F_c fragment (Jackson Immunoresearch Laboratories, Inc. West Grove, PA). After washing, the plates were incubated with the substrate, p-nitrophenyl phosphate disodium (Sigma) and read using an ELISA reader at 405 nm. Sera manifesting an O.D. reading, after subtraction of the background, of 0.3 or greater, were scored as positive.

For the immunoprecipitation assay, TCA-insoluble fraction of recombinant wild type or mutant p53 or lysates of Meth A tumor cells known to produce mutant p53 (Arai et al., 1986) metabolically labeled with ³⁵S-methionine, were reacted with antibodies or test sera for 2h at 4°C. The immune complexes were precipitated with Sepharose Protein A, separated on SDS-PAGE, electrotransferred to nitrocellulose membranes, and the proteins were detected using the Protoblot western blot Ap system (Promega, Madison, WI).

The results, summarized in Table 1, show that unimmunized control mice and mice immunized with control mAb 9E10 were not positive for anti-p53 antibodies. In contrast, mice immunized with anti-mutated p53 mAb 240 showed antibodies to both the mutated and native forms of p53. Mice immunized with anti-wild type p53 mAb 246 were positive for anti-p53 antibodies to wild type p53 but not to mutated p53. Analysis of the isotypes of the anti-p53 antibodies using the ELISA assay indicated that the antibodies included those of the IgG2a isotype regulated by T-helper 1 type cells that secrete interferon gamma (IFN- γ ; Elias et al., 1997).

Table 1. Mice immunized with anti-p53 mAb develop immune responses to p53.

<u>Immunogen</u>	<u>Antibody Assay</u>	
	<u>antigen</u>	<u>Incidence of positive mice</u>
None	p53	0/20
	mutated p53	0/20
mAb 9E10	p53	0/20
	mutated p53	0/20
mAb 240	p53	10/10*
	mutated p53	10/10*
mAb 246	p53	10/10*
	mutated p53	0/10
mAb 421	p53	10/10*
	mutated p53	10/10*

* p<0.01

5

Fig. 1 shows the retardation in gel of the p53-specific oligonucleotide sequence by anti-DNA antibodies developing only in the mice immunized with mAb 246, but not with mAb 240, mAb 421, mAb 9E10 or mAb R73. This result indicates that anti-DNA antibodies may recognize specific sequences of DNA.

Fig. 2 shows a precipitation assay using an extract of the Meth A tumor metabolically labeled with S³⁵. This tumor produces mutant p53. It can be seen that mAb 240 (anti-mutant p53) precipitated the mutant p53 band, while mAb 246 did not precipitate the mutant p53 band, but did precipitate wild-type p53 (not shown). The mice immunized with mAb 421 or with mAb 240 developed specific anti-p53 antibodies. The mice immunized with mAb 246 or with mAb 9E10 did not develop antibodies to mutant p53. Immunoprecipitation of recombinant p53 confirmed these results.

15

EXAMPLE 2. Immunized mice reject tumors.

Antibodies to tumor antigens have not been found to be effective in rejecting solid tumors (LoBuglio and Saleh, 1992; Mellstedt et al., 1991; Velders et al., 1995). To learn

20

whether immunization with anti-p53 mAb might influence in vivo the development of a tumor bearing a mutated form of the p53 molecule, groups of BALB/c female mice were immunized as above with either mAb 240, mAb 246, mAb 421 or mAb 9E10. The mice were challenged intradermally with 10^6 Meth A tumor cells 10 days after the boost. Non-immunized mice were also tested. The results in Table 2 are expressed as the incidence of the mice that rejected the tumor in comparison to the mice that did not reject the tumor.

Table 2. Immunization with anti-p53 mAbs activates rejection of Meth A sarcoma cells.

Immunogen	Incidence of Rejected Tumors
None	0/30
mAb 240	9/10*
mAb 246	9/10*
mAb 421	9/10*
mAb9E10	0/10

* $p < 0.01$ compared to non-immunized control mice (none).

Table 3 summarizes the antibody results and also shows that the mice that developed anti-p53 antibodies also rejected the Meth a tumor. The tumor rejection experiments have been repeated 4 times and have involved 30-40 mice per group. About 90% of the mice developing p53 immunity rejected their tumors. It is quite possible that T cells were involved in the rejection process. However, it has been shown that intact antibodies can enter living cells (Alarcon-Segovia et al., 1996; Madaio et al., 1996; Yanase et al., 1994; Ma et al., 1991) and it is conceivable that the anti-p53 antibodies themselves killed the tumor cells, possibly by inducing apoptosis (Madaio et al., 1996). It is also possible that the anti-p53 mAbs exerted an anti-tumor effect through antibody-dependent cytotoxicity (ADCC). The antibodies may also have acted in synergy with T cells (see Vasovic et al., 1997).

The growth of the Meth A tumor cells was inhibited in mice immunized with mAb 240, mAb 421 or mAb 246. In contrast to the protection induced by these mAbs, no protection was induced by immunization to control mAb 9E10. Thus, resistance to tumor challenge was associated with the anti-p53 mAbs.

It can thus be summarized from the above results that immunization with anti-p53 mAb molecules induces anti-p53 immunity and can induce anti-DNA antibodies. The anti-DNA antibodies can show specificity for DNA recognized by different p53 domains. The anti-p53 autoimmunity is associated with tumor rejection. These results are summarized in Table 3.

Table 3. Immunization to anti-p53 mAb molecules induces specific anti-DNA and anti-p53 immunity. The anti-p53 immunity is associated with tumor rejection.

Induced Reactivities				
mAb	Specificity	Anti-DNA specific for the p53 reactive sequence	anti-p53	Tumor rejection
9E10	Myc	No	No	No
240	mutated p53	No	Yes	Yes
246	p53-specific domain	Yes	Yes	Yes
421	p53-carboxy domain	No	Yes	Yes

Although antibodies naturally arising in tumor patients to the amino-terminal domain of p53 are not protective and may even indicate a poor prognosis (Schlichtholz et al., 1992), the rejection of tumors by mAb treatment may be explained by the fact that the mAbs were specific for other domains of p53. Moreover, the generation of IgG antibodies by the mAb treatment suggests that T cells may be involved. Be that as it may, the induction of anti-p53 reactivity by the use of specific mAbs is functionally effective.

EXAMPLE 3. Preparation of CDR-based peptides of anti-p53 mAbs

The light (L) and heavy (H) chains of the anti-p53 mAbs 240, 246, 248 and 421 were cloned and sequenced as described in Materials and Methods, section vii. The sequences are shown in Fig. 3 (CDR sequences are underlined).



Peptides V-IX described hereinabove were synthesized as described in Materials and Methods, section viii.

EXAMPLE 4. CDR-based peptides of anti-p53 mAbs induce anti-p53 immunity in mice

Immunization of BALB/c mice with peptides V-IX of Example 3 was carried out as described for the whole mAbs in Materials and Methods, section iv, using 100 µg of peptide, and then assayed for the development of anti-p53 antibodies.

The incidence of mice developing IgG anti-p53 antibodies (ELISA assay) was 8/10 and 7/10 for peptides V and VI, the CDR3-based peptides of mAb 240 and mAb 421, respectively.

The results of another experiment carried out with peptides V-IX (Table 4) show that mice immunized with these peptides rejected the Meth a tumor.

Table 4. Rejection of Meth A tumor in mice immunized with anti-p53 mAb CDR-based peptides V-IX

Peptide Immunogen	Incidence of rejected tumors
None	0/5
V	4/5
VI	5/5
VII	5/5
VIII	4/5
IX	4/5

These experiments show that fragments of anti-p53 mAb, e.g. CDR-based peptides, can be used to induce anti-p53 immunity.

EXAMPLE 5. Prevention of lung carcinoma metastasis by immunization with monoclonal antibodies to p53

Since the most likely application for immunotherapy of tumor is not only the treatment of solid tumors, but rather the treatment of metastasis after surgical removal of a solid tumor, the anti-p53 mAbs were also applied in a model of a metastasing tumor.

Immunization of C57BL/6 mice was performed with anti-p53 mAbs 240, 246, 421 and mAb R73 as control, as described in Materials and Methods, section (iv). Ten days after the boost, the mice were injected intradermally in the hind footpads with 2×10^5 3LL-lung carcinoma cells. Tumor growth at the injection site was observed. The tumors were excised when they reached 8 mm. The mice were sacrificed 21 days after tumor removal and their lungs were weighed as a quantitative measure of the metastatic load. In case of spontaneous mortality, the lung weight was determined on the day of death. Fig. 4 shows the protective effect of the p53 antibodies against the development of lung metastasis. All three anti-p53 mAbs tested significantly protected the 3LL-challenged mice against metastasis ($p < 0.01$, when compared to R73).

REFERENCES

1. Alarcon-Segovia, D., Ruiz-Arguelles, A. and Llorente, L. (1996). Broken dogma: penetration of autoantibodies into living cells. *Immunology Today* 17, 163-164.
2. Alzari, P.M., Lascombe, M.-B. and Poljak, R.J. (1988). Three-dimensional structure of antibodies. *Ann. Rev. Immunol.* 6, 555-80.
3. Arai, N., Nomura, D., Yokota, K., Wolf, D., Brill, E., Shohat, O., and Rotter, V. (1986). Immunologically distinct p53 molecules generated by alternative splicing. *Mol Cell Biol* 6, 3232-3239.
4. Bruggemann, M., Winter, G., Waldmann, H., and Neuberger, M. S. (1989). The immunogenicity of chimeric antibodies. *J. Exp. Med.* 170, 2153-2157.
5. Cazenave, P. A. (1977). Idiotypic-anti-idiotypic regulation of antibody synthesis in rabbits. *Proc Natl Acad Sci U S A* 74, 5122-5125.
6. Cohen, I. R. (1989). Natural Id-anti-Id networks and the immunological homunculus. In *Theories of Immune Networks*, H. Atlan and I. R. Cohen., ed. (Heidelberg: Springer-Verlag), pp. 6-12.



7. Cohen, I.R. (1992). The cognitive paradigm and the immunological homunculus. *Immunol Today* 13, 490-494.
8. Eisenbach, L., Segal, S. and Feldman, M. (1983). *Int J Cancer* 32, 113-120.
9. Elias, D., Meilin, A., Ablamunits, V., Birk, O.S., Carmi, P., Koenen-Waisman, S., and
5 Cohen, I.R. (1997). Hsp60 peptide therapy of NOD mouse diabetes induces a Th2 cytokine
burst and down-regulates autoimmunity to various B-cell antigens. *Diabetes* 46, 758-764.
10. el-Deiry, W. S., Kern, S. E., Pietenpol, J. A., Kinzler, K. W., and Vogelstein, B.
(1992). Definition of a consensus binding site for p53. *Nature Genet* 1, 45-49.
11. Gannon, J. V., Greaves, R., Iggo, R., and Lane, D. P. (1990). Activating mutations in
10 p53 produce a common conformational effect. A monoclonal antibody specific for the
mutant form. *Embo J* 9, 1595-1602.
12. Harlow, E., Crawford, L. V., Pim, D. C. and Williamson, N. M. (1981). Monoclonal
antibodies specific for simian virus 40 tumor antigens. *J Virol*, 39 861-869.
13. Hollstein, M., Sidransky, D., Vogelstein, B., and Harris, C. C. (1991). p53 mutations in
15 human cancers. *Science* 253, 49-53.
14. Houbiers, J. G., Nijman, H. W., van, d. B. S., Drijfhout, J. W., Kenemans, P., van, d.
V. C., Brand, A., Momburg, F., Kast, W. M., and Melief, C. J. (1993). In vitro induction of
human cytotoxic T lymphocyte responses against peptides of mutant and wild-type p53.
Eur J Immunol 23, 2072-2077.
- 20 15. Hunig, T., Wallny, H. J., Hartley, J. K., Lawetzky, A. and Tiefenthaler, G. (1989). *J*
Exp Med 169, 73-86.
16. Jerne, N. K. (1974). Towards a network theory of the immune system. *Ann. Imm.*
(Paris) 85, 2260-2264.
17. Lee, S., Elenbaas, B., Levine, A., and Griffith, J. (1995). p53 and its 14Kda C-terminal
25 domain recognize primary DNA damage in the form of insertion/deletion
18. LoBuglio, A. F., and Saleh, M. N. (1992). Advances in monoclonal antibody therapy
of cancer. *Am J Med Sci* 304, 214-224.
19. Lubin, R., Schlichtholz, B., Bengoufa, D., Zalcman, G., Tredaniel, J., Hirsch, A., de, F.
C., Preudhomme, C., Fenaux, P., Fournier, G., and et, a. l. (1993). Analysis of p53
30 antibodies in patients with various cancers define B-cell epitopes of human p53:
distribution on primary structure and exposure on protein surface. *Cancer Res* 53,
5872-5876.

20. Ma, J., Chapman, G. V., Chen, S. L., Melick, G., Pennie, R. and Breit, S. N.(1991). Antibody penetration of viable human cells. I. Increased penetration of human lymphocytes by anti-RNP IgG. *Clin exp Immunol* 84, 83-91.
21. Madaio, M. P., Fabbi, M., Tiso, M., Daga, A. and Puccetti, A. (1996). Spontaneously
5 produced anti-DNA/DNase I autoantibodies modulate nuclear apoptosis in living cells. *Eur J Immunol* 26, 3035-3041.
22. Melief, C. J., and Kast, W. M. (1991). T-cell immunotherapy of cancer. *Res Immunol* 142, 425-429.
23. Mellstedt, H., Frodin, J. E., Masucci, G., Ragnhammar, P., Fagerberg, J., Hjelm, A. L.,
10 Shetye, J., Wersall, P., and Osterborg, A. (1991). The therapeutic use of monoclonal antibodies in colorectal carcinoma. *Semin Oncol* 18, 462-477.
24. Moll, U. M., LaQuaglia, M., Benard, J., and Riou, G. (1995). Wild-type p53 protein undergoes cytoplasmic sequestration in undifferentiated neuroblastomas but not in differentiated tumors. *Proc Natl Acad Sci U S A* 92, 4407-4411.
- 15 25. Mozes, E., Dayan, M., Zisman, E., Brocke, S., Licht, A. and Pecht, I. (1989) *EMBO J.* 8, 4049.
26. Munro, S., and Pelham, H. R. (1986). An Hsp70-like protein in the ER: identity with the 78 kd glucose-regulated protein and immunoglobulin heavy chain binding protein. *Cell* 46, 291-300.
- 20 27. Nisonoff, A. (1991). Idiotypes: concepts and applications. *J. Immunol.* 147, 2429-2438.
28. Noguchi, Y., Chen, Y. T., and Old, L. J. (1994). A mouse mutant p53 product recognized by CD4+ and CD8+ T cells. *Proc Natl Acad Sci U S A* 91, 3171-3175.
29. Noguchi, Y., Richards, E. C., Chen, Y. T., and Old, L. J. (1995). Influence of
25 interleukin 12 on p53 peptide vaccination against established Meth A sarcoma. *Proc Natl Acad Sci U S A* 92, 2219-2223.
30. Old, L. J., Boyse, E. A., Clarke, D. A., and Carswell, E. (1962). *Ann. N.Y. Acad. Sci.* 101, 80-106.
- (Orlandi, R., Gussow, D.G., Jones P.T., and Winter, G. Cloning immunoglobulin variable domains for expression by the polymerase chain reaction. *Proc. Natl. Acad. Sci. USA*, 1989, 86:3833). (Sambrook, J., Fritsch, E.F. and Maniatis, T. *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Press, 1989).

31. Schlichtholz, B., Legros, Y., Gillet, D., Gaillard, C., Marty, M., Lane, D., Calvo, F., and Soussi, T. (1992). The immune response to p53 in breast cancer patients is directed against immunodominant epitopes unrelated to the mutational hot spot. *Cancer Res* 52, 6380-6384.
- 5 32. Shohat-Foord, O., Bhattacharya, P., Reich, Z., and Rotter, V. (1991). A DNA binding domain is contained in the C-terminus of wild type p53 protein. *Nucleic Acid Res.* 19, 5191-5198.
33. Soussi, T. (1996). The humoral response to the tumor suppressor gene product p53 in human cancer. Implications for diagnosis and therapy. *Immunol. Today* 17, 354-356.
- 10 34. Stevenson, F. K., Zhu, D., King, C. A., Ashworth, L. J., Kumar, S., and Hawkins, R. E. (1995). Idiotypic DNA vaccines against B-cell lymphoma. *Immunol. Rev.*, 211-228.
35. Takemori, T., Tesch, H., Reth, M., and Rajewsky, K. (1982). The immune response against anti-idiotope antibodies. I. Induction of idiotope-bearing antibodies and analysis of the idiotope repertoire. *Eur J Immunol* 12, 1040-1046.
- 15 36. Tilkin, A. F., Lubin, R., Soussi, T., Lazar, V., Janin, N., Mathieu, M. C., Lefrere, I., Carlu, C., Roy, M., Kayibanda, M., and et al. (1995). Primary proliferative T cell response to wild-type p53 protein in patients with breast cancer. *Eur J Immunol* 25, 1765-1769.
37. Urbain, J., Wikler, M., Franssen, J. D., and Collignon, C. (1977). Idiotypic regulation of the immune system by the induction of antibodies against anti-idiotypic antibodies. *Proc Natl Acad Sci U S A* 74, 5126-5130.
- 20 38. Vasovic, L.V., Dyal, R., Clynes, R.A., Ravetch, J.V., and Nikolic-Zugic (1997). Sinergy between an antibody and CD8⁺ cells in eliminating an established tumor. *Eur. J. Immunol.* 27, 374-382.
39. Velders, M. P., van Rhijn, C. M., Briaire, I. H., Fleuren, G. J., Warnaar, S. O., and Litvinov, S. V. (1995). Immunotherapy with low and high affinity monoclonal antibodies 17-1A and 323/A3 in a nude mouse xenograft carcinoma model. *Cancer Res* 55, 4398-4403.
- 25 40. Vile, R. and Russell, Stephen J. (1994) Gene transfer technologies for the gene therapy of cancer. *Gene Therapy* 1, 88-98.
- 30 41. Waisman, A., Mendlovic, S., Ruiz, P. J., Zinger, H., Meshorer, A., and Mozes, E. (1993). The role of the 16/6 idiotype network in the induction and manifestations of systemic lupus erythematosus. *Int Immunol* 5, 1293-1300.

42. Wettendorff, M., Koprowski, H. and Herlyn, D. 1990. Modulation of antitumor immunity by anti-idiotypic antibodies. in "Idiotypic Network and Diseases", eds. Cerny, J. and Hiernaux, J., American Society for Microbiology, Washington, D.C. pp 203-229.
43. Wolkowicz, R., Elkind, N. B., Ronen, D., and Rotter, V. (1995). The DNA binding
5 activity of wild type p53 is modulated by blocking its various antigenic epitopes. *Oncogene 10*, 1167-1174.
44. Yanase, K., Smith, R. M., Cizman, B., Foster, M. H., Peachey, L. D., Jarett, L. and Madaio, M. P. (1994). A subgroup of murine monoclonal anti-deoxyribonucleic acid
10 antibodies traverse the cytoplasm and enter the nucleus in a time- and temperature-dependent manner. *Lab Investigation 71*, 52-60.
45. Yanuck, M., Carbone, D. P., Pendleton, C. D., Tsukui, T., Winter, S. F., Minna, J. D., and Berzofsky, J. A. (1993). A mutant p53 tumor suppressor protein is a target for peptide-induced CD8+ cytotoxic T-cells. *Cancer Res 53*, 3257-3261
46. Yefenof, E., Picker, L. J., Scheuermann, R. H., Vitetta, E. S., Street, N. E., Tucker, T.
15 F., and Uhr, J. W. (1993). Induction of B cell tumor dormancy by anti-idiotypic antibodies. *Curr. Opinion Immunol. 5*, 740-744.
47. Yewdell, J. W., Gannon, J. V., and Lane, D. P. (1986). Monoclonal antibody analysis of p53 expression in normal and transformed cells. *J Virol 59*, 444-452.
48. Zambetti, G. P., and Levine, A. J. (1993). A comparison of the biological activities of
20 wild-type and mutant p53. *FASEB J 7*, 855-865.

CLAIMS:

1. Use of an immunogen selected from the group consisting of:

- (i) a monoclonal antibody (mAb) to a tumor-associated antigen (TAA);
- 5 (ii) a fragment of a mAb of (i);
- (iii) a peptide based on a complementarity-determining region (CDR) of the heavy or light chain of said mAb of (i), which peptide is capable of eliciting antibodies to said TAA; and
- (iv) a DNA molecule coding for the variable (V) region of said mAb of (i) in a
- 10 suitable gene delivery vehicle,

for the preparation of a pharmaceutical composition useful for induction of anti-tumor immunity in mammals, for activating an enhanced immune response to a TAA molecule in mammals, and/or for induction of immune responses to mutated and wild-type forms of a TAA in mammals.

15 2. The use according to claim 1, wherein the anti-TAA mAb is an anti-wild type or an anti-mutant TAA mAb.

3. The use according to claim 1, wherein the anti-TAA mAb fragment is selected from

20 antigen-binding fragments (Fab), F(ab')₂ or any other type of antibody molecule, including single chain Fv fragments, of anti-TAA mAb.

4. The use according to claim 1, wherein the immunogen is a peptide based on a CDR of the heavy or light chain of an anti-TAA mAb.

25 5. The use according to any one of claims 1 to 4, wherein the immunogen is an anti-p53 mAb, a fragment thereof, a peptide based on a CDR of the heavy or light chain of an anti-p53 mAb, or a DNA molecule coding for the variable (V) region of an anti-p53 mAb in a suitable gene delivery vehicle.

30 6. The use according to claim 5, wherein the anti-p53 mAb is an anti-wild type or an anti-mutant p53 mAb.

7. The use according to claim 5 or 6, wherein said peptide contains a sequence selected from:

(i) Peptides, herein designated Ia-Ib, based on the CDR2 and CDR3, respectively, of the heavy chain (240VH), and peptide Ic based on the CDR3 of the light chain (240VL), of the anti-p53 mAb 240, of the sequences:

(Ia) Glu-Ile-Asp-Pro-Ser-Asp-Ser-Tyr-Thr-

Asn-Tyr-Asn-Gln-Asn-Phe-Lys-Asp

(Ib) Leu-Leu-Arg-Tyr-Phe-Ala-Met-Asp-Tyr

10 (Ic) Gln-His-Ile-Arg-Glu-Leu-Thr-Arg

(ii) Peptides, herein designated IIa-IIb, based on the CDR2 and CDR3, respectively, of the heavy chain (246VH), and peptide IIc based on the CDR3 of the light chain (246VL), of the anti-p53 mAb 246, of the sequences:

(IIa) Asp-Ile-Asn-Pro-Asn-Asn-Gly-Tyr-Thr-

15 Ile-Tyr-Asn-Gln-Lys-Val-Lys-Gly

(IIb) Gly-Gly-Gly-Leu-Lys-Gly-Tyr-Pro-Phe-Val-Tyr

(IIc) Gln-Gln-Arg-Ser-Ser-Phe-Pro-Phe-Thr

(iii) Peptides, herein designated IIIa-IIIb, based on the CDR2 and CDR3, respectively, of the heavy chain (248VH), and peptide IIIc based on the CDR3 of the light chain (248VL), of the anti-p53 mAb 248, of the sequences:

20 (IIIa) Asp-Ile-Tyr-Pro-Asn-Asn-Gly-Phe-Thr-

Thr-Tyr-Asn-Gln-Lys-Phe-Lys-Gly

(IIIb) Ser-Gly-Ser-Arg-Phe-Asp-Tyr

(IIIc) Gln-Gln-Ser-Asn-Ser-Trp-Pro-Val-His-Ala

25 (iv) Peptides, herein designated IVa-IVb, based on the CDR2 and CDR3, respectively, of the heavy chain (421VH), and peptide IVc based on the CDR3 of the light chain (421VL), of the anti-p53 mAb 421, of the sequences:

(IVa) Trp-Ile-Asp-Pro-Glu-Asn-Gly-Asp-Thr-

Glu-Tyr-Ala-Pro-Lys-Phe-Gln-Gly

30 (IVb) Tyr-Gly-Asp-Ala-Leu-Asp-Tyr

(IVc) Trp-Gln-Gly-Thr-His-Ser-Pro-Leu-Thr

8. The use according to claim 7, wherein said peptide contains a sequence selected from the sequences Ic, IIa, IIIb, IIIc and IVc.

9. The use according to claim 8 of the peptides V-IX of the sequences:

- 5 Peptide V: Tyr-Tyr-Cys-Gln-His-Ile-Arg-Glu-
 Leu-Thr-Arg-Ser-Glu-Gly-Gly-Pro-Ser
- Peptide VI: Gly-Val-Tyr-Tyr-Cys-Trp-Gln-Gly-Thr-His-
 Ser-Pro-Leu-Thr-Phe-Gly-Ala-Gly-Thr-Lys
- Peptide VII: Gly-Asp-Ile-Asn-Pro-Asn-Asn-Gly-Tyr-Thr-
10 Ile-Tyr-Asn-Gln-Lys-Val-Lys-Gly-Lys-Ala
- Peptide VIII: Ala-Val-Tyr-Tyr-Cys-Ala-Arg-Ser-Gly-Ser-
 Arg-Phe-Asp-Tyr-Trp-Gly-Glu-Gly-Thr-Thr
- Peptide IX: Val-Tyr-Phe-Cys-Gln-Gln-Ser-Asn-Ser-Trp-
 Pro-Val-His-Ala-Arg-Gly-Gly-Gly-Thr-Lys

15

10. A method for induction of anti-tumor immunity in a mammal comprising administering to said mammal an effective amount of at least one immunogen selected from the group consisting of:

- (i) a mAb to a TAA;
- 20 (ii) a fragment of said mAb of (i);
- (iii) a peptide based on a CDR of the heavy or light chain of said mAb of (i),
 which peptide is capable of eliciting antibodies to said TAA; and
- (iv) a DNA molecule coding for the variable (V) region of the mAb of (i) in a
 suitable gene delivery vehicle.

25

11. A method for activating an enhanced immune response to a TAA antigen in a mammal comprising immunizing said mammal with an effective amount of at least one immunogen selected from the group consisting of :

- (i) a mAb to a TAA;
- 30 (ii) a fragment of said mAb of (i);
- (iii) a peptide based on a CDR of the heavy or light chain of said mAb of (i),
 which peptide is capable of eliciting antibodies to said TAA; and

- (iv) a DNA molecule coding for the variable (V) region of the mAb of (i) in a suitable gene delivery vehicle.

12. A method for induction of immune responses to mutated and wild-type forms of a TAA in a mammal comprising immunization of said mammal with an effective amount of at least one immunogen selected from the group consisting of:

- (i) a mAb to a TAA;
(ii) a fragment of said mAb of (i);
(iii) a peptide based on a CDR of the heavy or light chain of said mAb of (i), which peptide is capable of eliciting antibodies to said TAA; and
(iv) a DNA molecule coding for the variable (V) region of the mAb of (i) in a suitable gene delivery vehicle.

13. The method according to any one of claims 10-12, wherein the immunogen is selected from the group consisting of an anti-p53 mAb, a fragment thereof, a peptide based on a CDR of the heavy or light chain of an anti-p53 mAb, or a DNA molecule coding for the variable (V) region of an anti-p53 mAb in a suitable gene delivery vehicle.

14. The method according to claim 13, wherein the anti-p53 mAb is selected from the group consisting of murine, human and humanized anti-wild type p53 and anti-mutant p53 mAb.

15. The method according to claim 13, wherein the immunogen is an anti-p53 mAb fragment selected from antigen-binding fragments (Fab), F(ab')₂ or any other type of antibody molecule, including single chain Fv fragments, of anti-p53 mAb.

16. The method according to claim 13, wherein the immunogen is a peptide based on a CDR of the heavy or light chain of an anti-p53 mAb.

17. The method according to claim 16, wherein the peptide contains a sequence of the CDR2 or CDR3 of the heavy chain, or of the CDR3 of the light chain, of an anti-p53 mAb selected from mAb 240, 246, 248 and 421, as follows:



(i) Peptides, herein designated Ia-Ib, based on the CDR2 and CDR3, respectively, of the heavy chain (240VH), and peptide Ic based on the CDR3 of the light chain (240VL), of the anti-p53 mAb 240, of the sequences:

(Ia) Glu-Ile-Asp-Pro-Ser-Asp-Ser-Tyr-Thr-

5 Asn-Tyr-Asn-Gln-Asn-Phe-Lys-Asp

(Ib) Leu-Leu-Arg-Tyr-Phe-Ala-Met-Asp-Tyr

(Ic) Gln-His-Ile-Arg-Glu-Leu-Thr-Arg

(ii) Peptides, herein designated IIa-IIb, based on the CDR2 and CDR3, respectively, of the heavy chain (246VH), and peptide IIc based on the CDR3 of the light chain (246VL), of the anti-p53 mAb 246, of the sequences:

(IIa) Asp-Ile-Asn-Pro-Asn-Asn-Gly-Tyr-Thr-

Ile-Tyr-Asn-Gln-Lys-Val-Lys-Gly

(IIb) Gly-Gly-Gly-Leu-Lys-Gly-Tyr-Pro-Phe-Val-Tyr

(IIc) Gln-Gln-Arg-Ser-Ser-Phe-Pro-Phe-Thr

(iii) Peptides, herein designated IIIa-IIIb, based on the CDR2 and CDR3, respectively, of the heavy chain (248VH), and peptide IIIc based on the CDR3 of the light chain (248VL), of the anti-p53 mAb 248, of the sequences:

(IIIa) Asp-Ile-Tyr-Pro-Asn-Asn-Gly-Phe-Thr-

Thr-Tyr-Asn-Gln-Lys-Phe-Lys-Gly

20 (IIIb) Ser-Gly-Ser-Arg-Phe-Asp-Tyr

(IIIc) Gln-Gln-Ser-Asn-Ser-Trp-Pro-Val-His-Ala

(iv) Peptides, herein designated IVa-IVb, based on the CDR2 and CDR3, respectively, of the heavy chain (421VH), and peptide IVc based on the CDR3 of the light chain (421VL), of the anti-p53 mAb 421, of the sequences:

25 (IVa) Trp-Ile-Asp-Pro-Glu-Asn-Gly-Asp-Thr-

Glu-Tyr-Ala-Pro-Lys-Phe-Gln-Gly

(IVb) Tyr-Gly-Asp-Ala-Leu-Asp-Tyr

(IVc) Trp-Gln-Gly-Thr-His-Ser-Pro-Leu-Thr

30 18. The method according to claim 17, wherein said peptide contains a sequence selected from the sequences Ic, IIa, IIIb, IIIc and IVc.

19. The method according to claim 18, wherein the peptides are selected from peptides V-IX of the sequences:

- Peptide V: Tyr-Tyr-Cys-Gln-His-Ile-Arg-Glu-
Leu-Thr-Arg-Ser-Glu-Gly-Gly-Pro-Ser
- 5 Peptide VI: Gly-Val-Tyr-Tyr-Cys-Trp-Gln-Gly-Thr-His-
Ser-Pro-Leu-Thr-Phe-Gly-Ala-Gly-Thr-Lys
- Peptide VII: Gly-Asp-Ile-Asn-Pro-Asn-Asn-Gly-Tyr-Thr-
Ile-Tyr-Asn-Lys-Val-Lys-Gly-Lys-Ala
- Peptide VIII: Ala-Val-Tyr-Tyr-Cys-Ala-Arg-Ser-Gly-Ser-
10 Arg-Phe-Asp-Tyr-Trp-Gly-Glu-Gly-Thr-Thr
- Peptide IX: Val-Tyr-Phe-Cys-Gln-Gln-Ser-Asn-Ser-Trp-
Pro-Val-His-Ala-Arg-Gly-Gly-Gly-Thr-Lys

20. The method according to any one of claims 10-12, which comprises administering to
15 a patient effective amounts of two or more different mAbs directed to the same TAA or to
different TAAs, or fragments thereof, either concomitantly or sequentially at different
intervals.

21. The method according to any one of claims 10-12, which comprises administering to
20 a patient effective amounts of two or more different peptides based on the same or
different CDRs of the same mAb or of different mAbs directed to the same TAA or to
different TAAs, either concomitantly or sequentially at different intervals.

22. Synthetic peptides containing a sequence based on a CDR of the heavy or light chain
25 of an anti-TAA mAb, and salts and chemical derivatives thereof.

23. Synthetic peptides according to claim 22, wherein the peptide contains a sequence
based on a CDR of the heavy or light chain of an anti-p53 mAb, and salts and chemical
derivatives thereof.

30

24. Synthetic peptides according to claim 23, wherein the peptide contains a sequence of the CDR2 or CDR3 of the heavy chain, or of the CDR3 of the light chain, of an anti-p53 mAb selected from mAb 240, 246, 248 and 421, as follows:

(i) Peptides, herein designated Ia-Ib, based on the CDR2 and CDR3, respectively, of the heavy chain (240VH), and peptide Ic based on the CDR3 of the light chain (240VL), of the anti-p53 mAb 240, of the sequences:

(Ia) Glu-Ile-Asp-Pro-Ser-Asp-Ser-Tyr-Thr-

Asn-Tyr-Asn-Gln-Asn-Phe-Lys-Asp

(Ib) Leu-Leu-Arg-Tyr-Phe-Ala-Met-Asp-Tyr

10 (Ic) Gln-His-Ile-Arg-Glu-Leu-Thr-Arg

(ii) Peptides, herein designated IIa-IIb, based on the CDR2 and CDR3, respectively, of the heavy chain (246VH), and peptide IIc based on the CDR3 of the light chain (246VL), of the anti-p53 mAb 246, of the sequences:

(IIa) Asp-Ile-Asn-Pro-Asn-Asn-Gly-Tyr-Thr-

15 Ile-Tyr-Asn-Gln-Lys-Val-Lys-Gly

(IIb) Gly-Gly-Gly-Leu-Lys-Gly-Tyr-Pro-Phe-Val-Tyr

(IIc) Gln-Gln-Arg-Ser-Ser-Phe-Pro-Phe-Thr

(iii) Peptides, herein designated IIIa-IIIb, based on the CDR2 and CDR3, respectively, of the heavy chain (248VH), and peptide IIIc based on the CDR3 of the light chain (248VL), of the anti-p53 mAb 248, of the sequences:

(IIIa) Asp-Ile-Tyr-Pro-Asn-Asn-Gly-Phe-Thr-

Thr-Tyr-Asn-Gln-Lys-Phe-Lys-Gly

(IIIb) Ser-Gly-Ser-Arg-Phe-Asp-Tyr

(IIIc) Gln-Gln-Ser-Asn-Ser-Trp-Pro-Val-His-Ala

25 (iv) Peptides, herein designated IVa-IVb, based on the CDR2 and CDR3, respectively, of the heavy chain (421VH), and peptide IVc based on the CDR3 of the light chain (421VL), of the anti-p53 mAb 421, of the sequences:

(IVa) Trp-Ile-Asp-Pro-Glu-Asn-Gly-Asp-Thr-

Glu-Tyr-Ala-Pro-Lys-Phe-Gln-Gly

30 (IVb) Tyr-Gly-Asp-Ala-Leu-Asp-Tyr

(IVc) Trp-Gln-Gly-Thr-His-Ser-Pro-Leu-Thr

25. Synthetic peptides according to claim 24, wherein the peptide contains a sequence selected from the sequences Ic, IIa, IIIb, IIIc and IVc.

26. Synthetic peptides according to claim 25, wherein the peptides are selected from peptides V-IX of the sequences:

- Peptide V: Tyr-Tyr-Cys-Gln-His-Ile-Arg-Glu-
Leu-Thr-Arg-Ser-Glu-Gly-Gly-Pro-Ser)
- Peptide VI: Gly-Val-Tyr-Tyr-Cys-Trp-Gln-Gly-Thr-His-
Ser-Pro-Leu-Thr-Phe-Gly-Ala-Gly-Thr-Lys
- 10 Peptide VII: Gly-Asp-Ile-Asn-Pro-Asn-Asn-Gly-Tyr-Thr-
Ile-Tyr-Asn-Gln-Lys-Val-Lys-Gly-Lys-Ala
- Peptide VIII: Ala-Val-Tyr-Tyr-Cys-Ala-Arg-Ser-Gly-Ser-
Arg-Phe-Asp-Tyr-Trp-Gly-Glu-Gly-Thr-Thr
- 15 Peptide IX: Val-Tyr-Phe-Cys-Gln-Gln-Ser-Asn-Ser-Trp-
Pro-Val-His-Ala-Arg-Gly-Gly-Gly-Thr-Lys

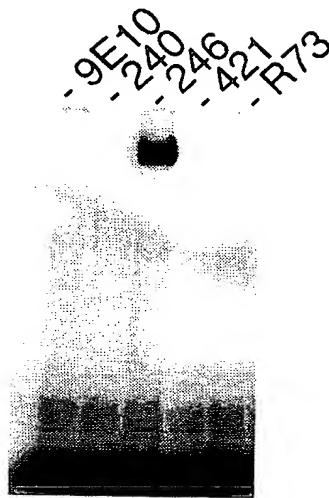
27. A method for the generation of sequence-specific anti-DNA antibodies which comprises immunizing a mammal with a mAb directed to a domain containing a DNA-binding site of a DNA-binding protein, and recovering the thus elicited
20 sequence-specific anti-DNA antibodies.

28. The method according to claim 27, wherein the DNA-binding protein is p53.

29. The method according to claim 28, wherein the mAb is directed against the central
25 DNA-binding domain of the p53 molecule.

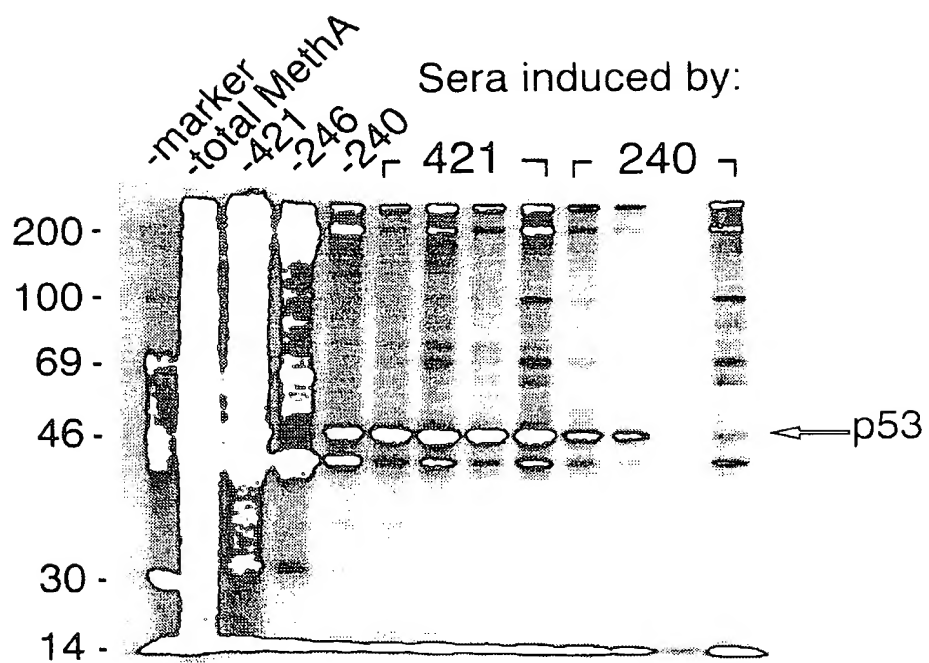
30. The method according to claim 29, wherein the anti-p53 mAb is the mAb 246.

Figure 1



2/5

Figure 2A



3/5

Figure 2B

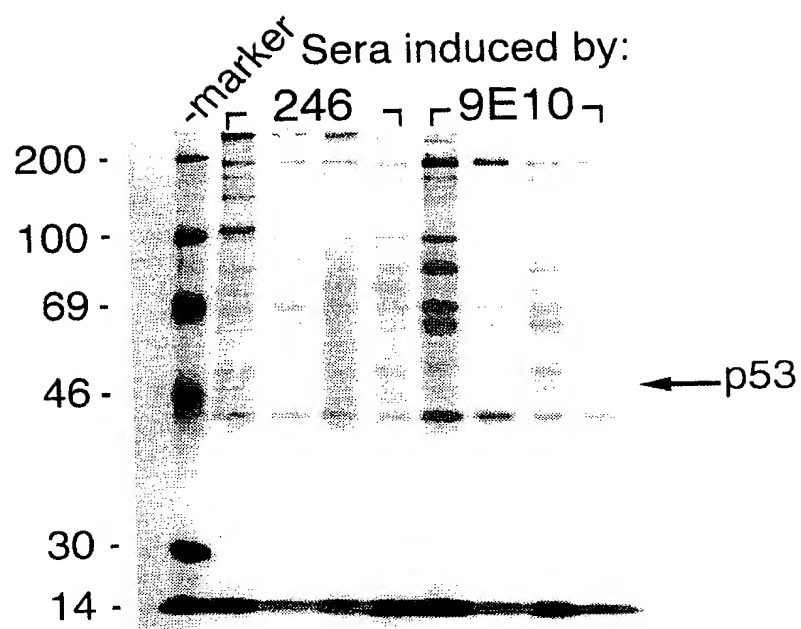


Figure 3:

240 VH:

ATGEAGASVKLSCKASGYTFTSSWINWVKQRPGGLEWIGEIDPSDSYTN
YNQNFKDKATLTVDKSSSTAYMQLSSLTSEDSAVYYCARLLRYFAMDYWG
QGTT

246 VH:

SWVKPGASVKIPCKASGYTFTDYNMDWVKQSHGKSLEWIGDINPNNGYTI
YNQKVKGKATLTVDKSSNTAYMELRSLTSEDTAVYYCVRGGGLKGYPFVY
WGQGT

248 VH:

LQESGPELVKPGASVKISCKASGYTFTDYYMNWVKQSHGKSLEWIGDIYPN
NGFTTYNQKFKGKATLTVDKSSSTAYNELRNLTSEDSAVYYCARSGSRFD
YWGQGTTVHR

421 VH:

LQESGAELVRSGASVKLSCTASGFNIKDYYMHWVKQRPEQGLEWIGWIDP
ENGDTEYAPKFQGKATMTADTSSNTAYLQLSSLASEDTAVYYCNFYGDAL
DYWGQGTTVTVS

240 VL:

FLSCISGAEGHHLIQGGQKCQYIWLYMHWNQQKPGQPPRLLIYLVSNLE
SGVPARFSGSGSGTDFTLNIHPVEEEDAATYYCQHIRELTRSEGGPSWRSI
RI

246 VL:

KVTITCSASSSVNFMHWFQQKPGTSPKLWIYSTSNLASGVPARFIGSGSGT
S?SLTISRMEAEHAATYYCQQRSSFPFTYGSGTK?EIQ?DP

248 VL:

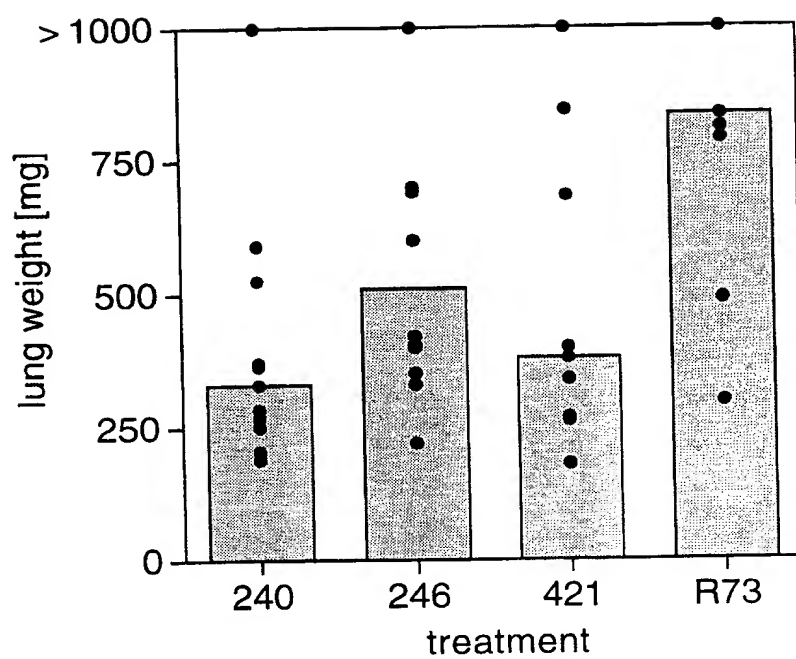
MDIQLTQSPATLSVTPGDSVSLSCRASQSISNNLHWYQQKSHESPRLLIKE
ASQSISGIPSRFSGSGSGTDFTLSINSVETEDFGVYFCQQSNSWPVHARG
GGTKLENL

421 VL:

PQDIQLTQSPLTSLVTIGQPASISCKSSQSLLDSDGKTYLNWLLQRPGQSP
KRLIYLVSKLDSGVPDRFTGSGSGTDFTLKINRVEAEDLGVYYCWQGTHSP
LTFGAGTKLK

5/5

Figure 4



INTERNATIONAL SEARCH REPORT

Application No

PCT/IL 98/00266

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K39/395 A61K48/00 C07K16/30 C07K16/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
------------	--	-----------------------

X	<p>WO 93 18792 A (BIOMIRA INC) 30 September 1993</p> <p>see page 4, line 15 - page 5, line 6 see page 6, line 24 - page 7, line 13 see page 15, line 5-35 see table 1</p>	<p>1-4, 10-12, 20-22</p>
Y	<p>---</p> <p>-/--</p>	<p>5-9, 13-19, 27-30</p>

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

26 October 1998

Date of mailing of the international search report

11/11/1998

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Covone, M

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/IL 98/00266

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO 96 01126 A (IMMUNOMEDICS INC) 18 January 1996</p> <p>see page 2, line 32 - page 3, line 10 see page 7, line 15-26 see page 15, line 1 - page 16, line 24 see examples 2-4 see claim 21</p>	<p>1-4, 10-12, 20-22</p>
Y	<p>--- ZUSMAN I. ET AL.: "Tumor-suppressor effets of anti-p53 IgG on chemically induced colon cancer in rats" THE CANCER JOURNAL, vol. 10, no. 2, April 1997, pages 116-120, XP002081863 see page 116, left-hand column, line 14-25 see page 117, right-hand column, line 41-46 see section: Discussion</p>	<p>5-9, 13-19</p>
X	<p>--- NICHOLSON S. ET AL.: "Anti-tumor immune responses following monoclonal antibody therapy of ovarian cancer" PROC.ANNU.MEET.AM.ASSOC.CANCER RES, vol. 38, March 1997, XP002081861 A2702 see the whole document</p>	<p>1-4, 10-12, 20-22</p>
X	<p>--- GANNON J V ET AL: "Activating mutations in p53 produce a common conformational effect. A monoclonal antibody specific for the mutant form" EMBO JOURNAL, vol. 9, no. 5, March 1990, pages 1595-1602, XP000676347 cited in the application see page 1595, right-hand column, line 52 - page 1596, left-hand column, line 9 see page 1601 "Antibodies"</p>	<p>22-26</p>
X	<p>--- WO 97 04092 A (RHONE POULENC RORER SA ;CONSEILLER EMMANUEL (FR); BRACCO LAURENT () 6 February 1997 see page 88-90 see claim 36</p>	<p>22-26</p>
X	<p>--- JANNOT C B ET AL: "Characterization of scFv-421, a single-chain antibody targetd to p53" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 230, no. 2, 13 January 1997, pages 242-246, XP002035502 see figure 4</p>	<p>22-26</p>

-/--

INTERNATIONAL SEARCH REPORT

Application No
PCT/IL 98/00266

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WOLKOWICZ R. ET AL. : "The DNA binding activity of wild type p53 is modulated by blocking its various antigenic epitopes" ONCOGENE, vol. 10, 1995, pages 1167-1174, XP002081864 see abstract see pages 1170-1171 section "PAb-246, PAb-1620 and PAb-200.47 monoclonal..." see page 1172, right-hand column, line 51-65	22-26
Y	---	27-30
X	WO 92 13970 A (ONCOGENE SCIENCE INC) 20 August 1992 see page 18, line 5-10 see page 21, line 23-24 see page 45, line 11-13 ---	22-26
P,X	WO 98 18825 A (DEBUSSCHE LAURENT ;BRACCO LAURENT (FR); RHONE POULENC RORER SA (FR) 7 May 1998 see page 31 see claim 5 ---	22-26
P,X	RUIZ P.J. ET AL.: "Idiotypic immunization induces immunity to mutated p53 and tumor rejection" NATURE MEDICINE, vol. 4, no. 6, June 1998, pages 710-712, XP002081865 see the whole document -----	1-27

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL 98/00266

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 10-21 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Application No
PCT/IL 98/00266

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9318792 A	30-09-1993	AU 3881593 A CA 2131621 A EP 0631507 A FI 944314 A JP 7504668 T NO 943388 A	21-10-1993 30-09-1993 04-01-1995 16-09-1994 25-05-1995 18-11-1994
WO 9601126 A	18-01-1996	US 5798100 A AU 2953895 A CA 2194166 A EP 0768894 A JP 10503758 T	25-08-1998 25-01-1996 18-01-1996 23-04-1997 07-04-1998
WO 9704092 A	06-02-1997	FR 2736915 A AU 6618696 A CA 2224468 A CZ 9800144 A EP 0839194 A NO 980203 A	24-01-1997 18-02-1997 06-02-1997 15-04-1998 06-05-1998 10-03-1998
WO 9213970 A	20-08-1992	AU 1370592 A EP 0576476 A	07-09-1992 05-01-1994
WO 9818825 A	07-05-1998	FR 2755144 A AU 4952097 A	30-04-1998 22-05-1998



THIS PAGE BLANK (USPTO)